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INTRA– AND INTERSPECIFIC DIFFERENCES IN RATE OF OXYGEN CONSUMPTION IN GOBIID FISHES OF THE GENUS GILLICHTHYS [1]

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Many investigators have searched for adaptive physiological differences between intraspecific units. The growing literature has been reviewed a number of times (Thorpe, 1940; Timofeef-Ressovsky, 1940; Dobzhansky, 1951, Ch. 6; Prosser, 1955, 1957; Dehnel, 1955; Moore, 1957).

Recently attention has turned to physiological variation in relation to latitudinal distribution (Roscoff, Station Biologique, 1957; Vernberg 1959a, 1959b; Vernberg and Tashian, 1959). The results from most of the past research of this type cannot be applied to studies of intraspecific divergence (Prosser, 1957). Unfortunately, the early workers either did not consider thermal acclimation (Bullock, 1955, for review), or purposely acclimated the animals to the temperatures at which they were found (*e.g.,* Rao, 1953). Thus latitudinal and acclimation effects have not been clearly separable. Developmental rates of embryos from latitudinally separated populations (Dehnel, 1955) may be independent of latitudinal difference in environment, but only if temperature at the time of yolk formation is without effect on the metabolism of the yolk-consuming embryo. To appreciate genetically adaptive divergence in physiology as a function of latitude, the animals must be acclimated to, and compared at, the same temperatures.

The present study was undertaken to establish the degree of physiological divergence, if any, between widely separated populations of *Gillichthys mirabilis* Cooper. The results of an extensive morphological examination of several populations of this species, and of *Gillichthys seta* (Ginsburg), (Barlow, 1961, and unpublished data) constitute the point of departure for the program at hand. Oxygen consumption, a highly integrated metabolic phenomenon, was the physiological system chosen for the investigation.

MATERIAL AND METHODS

Gillichthys seta doubtless is the species most closely related to *G. mirabilis* (Barlow, 1961). In occurrence, *G. seta* is restricted to the northern part of the Gulf of California. It is found only along the exposed coast in the highest intertidal zone among the streams and pools of rocky reefs.

The habitat of *G. mirabilis* is the intertidal region of coastal sloughs. Typically, this is a muddy region exposed to air at low tide. Some of the fish remain at low tide in crab burrows, or the likes, and breathe air; *G. seta* also is capable of aerial respiration.

The distribution of *G. mirabilis* embraces several faunal regions. The northmost populations in central California co-exist with a low-boreal fauna; the southmost populations in the Gulf of California occur within a tropical Panamic region; and the northern part of the Gulf of California, where *G. mirabilis* is also found, has a depauperate, mixed fauna. Thus *G. mirabilis* is arranged in disjunct populations over three roughly parallel, north-south, coast lines.

Distribution along a north-south coast ordinarily corresponds to a temperature gradient, warmer to the south. This applies to the Pacific Coast, and in the winter to the Gulf of California. In the late summer, however, the gradient in the Gulf is either reversed, cooler to the south, or the temperatures are everywhere about the same. As a result, the annual range of mean temperatures in the Gulf of California increases from south to north. The climate of the Salton Sea continues this trend of increased temperature extremes to the north.

In 1930 *G. mirabilis* was introduced into the Salton Sea. The parental stock came from San Diego Bay, just a few miles south of the slough at Los Peñasquitos. The fish have become well established in the Salton Sea in spite of the many peculiar features (Carpelan, 1958) of this environment. Judging from the number of aberrant specimens, the Salton Sea is a marginal habitat for *G. mirabilis*.

Treatment of the animals

The fish were held in sea water in 20- and 40-gallon tanks. The water was aerated, and filtered continuously, and was replaced with fresh sea water two to three days prior to each experiment.

The fish were fed exclusively on fresh squid, which they ate voraciously. The number of days between feedings, as well as the feeding time, were purposely kept irregular to minimize cyclic behavior. They were not fed just prior to an experiment; this interval was adjusted according to the size of the fish and the acclimation temperature (at 10°, intervals of seven to twelve days, and at 24°, of two to four days; longer periods for longer fish), so that the fish when tested were flat-bellied, and production of fecal material was low.

The minimum acclimation time was two weeks at 24° ± 0.5°, and four weeks at 10° ± 0.05°. Experience indicated acclimation was complete sooner than that, especially at 24°. In changing fish from one temperature to the other, they were held for one week at the intermediate temperature of 17°, as was done in the experiments by Brett (1952). Illumination was continuous. For both species, only male or immature fish were used.

Apparatus

Special chambers for the Warburg apparatus were constructed, from ground-glass fittings, to accommodate the elongate fish, and in various sizes (45-cc., 85-cc., and 250-cc.) to maintain the proper ratio between fish and chamber volumes.

Reduction of the oxygen tension in the chamber was circumvented by installing an oxygen well, a collapsible bag made of No. 6, 100 gauge, Saran film, on the air inlet of the manometer. Each time the stopcock was opened to balance the manometer fluid, pure oxygen, at atmospheric pressure, replaced that utilized.

Experimental procedure

Each fish was blotted gently, then weighed in the chamber. Filtered sea water was added in an exact amount, equal to about 5% of the flask volume (enough water to wet the belly and the floor of the mouth of the fish). The flask was then flushed with oxygen, potassium hydroxide solution was quickly introduced into the cup, and the flask was affixed to the manometer. The bath was darkened and the manometers were stationary. Temperatures were controlled to within $\pm 0.025°$. The initial temperature was always the same as the acclimation temperature of the fish.

The animals were placed in the bath in the evening, and the first reading was taken twelve hours later between 0830 and 0930 hours. To determine the adjustment period, however, readings were commenced 30 minutes after placing the fish in the bath.

Oxygen uptake was recorded at the acclimation temperature during the thirteenth and fourteenth hours in the chamber. The bath temperature was then steadily raised 7° during one hour. Two hours elapsed at the new temperature before the oxygen uptake was recorded during another two-hour period, the eighteenth and nineteenth hours in the flasks.

Excepting the fish from Puerto San Carlos, no animal was used a second time in the same series of acclimation experiments.

The respiratory rates were compared at 10° and 17° for four populations of *G. mirabilis* acclimated to 10°. From north to south, the fish were from San Francisco Bay (Alviso Ponds), latitude 37° N., in central California; Los Peñasquitos, latitude 33° N., in southern California; the Salton Sea, latitude 33° N.; and San Felipe ("El Marino" slough), latitude 31° N., in the northern part of the Gulf of California. Though the Salton Sea and Los Peñasquitos are on about the same latitude, the warmer climate of the Salton Sea is considered here to be more "southern." San Felipe is not much farther south than southern California but is in a region with a warmer over-all climate.

Fish from the same four places were also acclimated to 24° and tested at 24° and 31°. A group of fish from Puerto San Carlos, latitude 28° N., near Guaymas in the central part of the Gulf of California, was included in the latter comparison. The *G. mirabilis* from San Carlos were small when obtained. In spite of growing well in aquaria, the distribution of sizes of the San Carlos fish included much smaller animals than in the other samples. Consequently they were not included in the analysis of co-variance within the species *G. mirabilis*. Comparisons between *G.*

mirabilis and *G. seta* were based on experiments with *G. mirabilis* from San Carlos, and *G. seta* from Puertecitos, latitude 30° N.

Treatment of the data

The decision to accept or reject the stated statistical hypothesis was based on the 5% critical level in order to minimize type II errors (Hoel, 1954, p. 33). Acceptance of a hypothesis such as equal means does not imply the populations in question were proven to be the same.

The mean rate of oxygen consumption was employed throughout. Minimum rates were only 3% to 5% below the mean.

Regression lines were fitted by least squares to the logarithms of the experimental data. It was convenient to express this rectilinear relationship as oxygen consumption per unit weight, instead of per total weight. The slope is then negative, as indicated in the expression: Rate $= a \, Wt^{-b}$, where a is the rate when weight (Wt) equals one, and b is the regression coefficient.
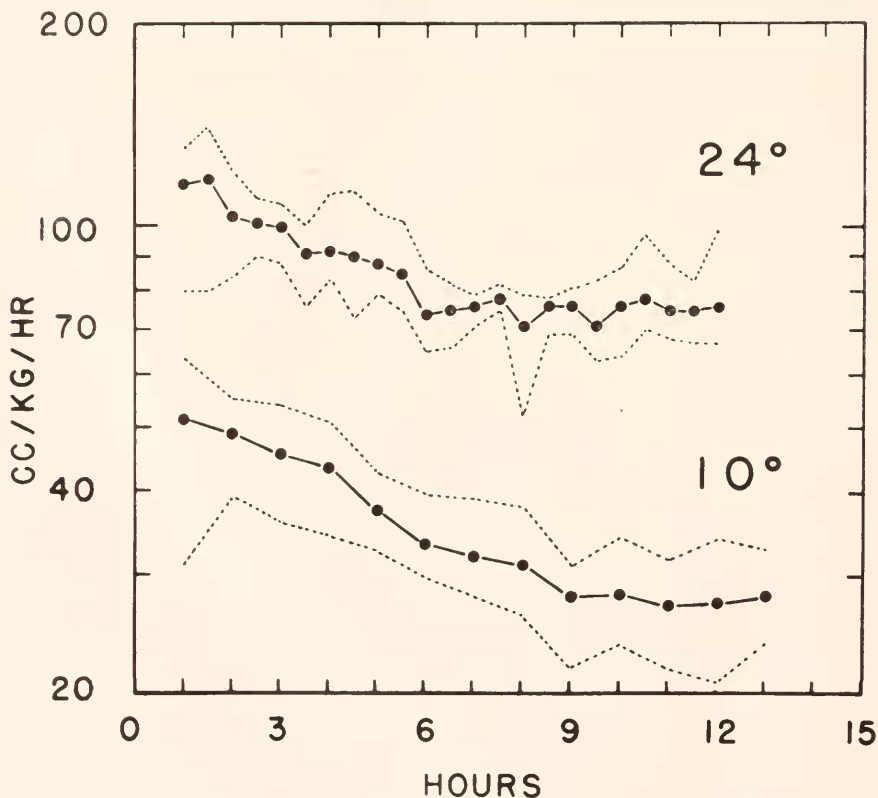

FIGURE 1. Time to steady rate of oxgen consumption for *Gillichthys mirabilis* from Los Peñasquitos. Solid lines connect means and dashed lines connect ranges. Individual rates were adjusted to the eight-gram intercept using regression coefficients of −0.28 for the 10° group and −0.56 for the 24° group (n = 5); the procedure is discussed in Roberts (1957).

<center>RESULTS</center>

Uniformity of results

The rate of oxygen consumption remained the same from month to month in a given population. Fish from the Salton Sea, tested in January, April, and May, showed no perceptible differences. And fish from San Carlos re-tested after periods of growth, continued to manifest rates appropriate to their earlier determined regression lines (Fig. 4).

The oxygen added to the chambers at the beginning of each experiment apparently did not disturb the fish. Controls with air, instead of oxygen, metered into the chambers showed no differences.

At 10° the initial rate of oxygen uptake was high, but reached a constant level within nine to ten hours (Fig. 1). The adjustment time at this temperature was independent of the size of the fish relative to the volume of the chamber.

The initial rate was also high at 24°. For all fish in small or medium size chambers, adjustment was achieved within six to ten hours (Fig. 1). The adjustment time in the largest chambers, however, varied depending on the ratio of the fish size to chamber volume. In the large chambers oxygen consumption was reasonably regular within eight to ten hours if the fish were larger than 15 grams. Because of this, only fish equal to or larger than 20 grams were used in large chambers at 24°.

Wells (1935a) investigated the adjustment time of *G. mirabilis,* but in water instead of air. The results of his four experiments show excellent agreement with

<center>TABLE I</center>

<center>*Relation of oxygen consumption to body weight in* Gillichthys mirabilis *and* G. seta</center>

Temperature	10° C.			17° C.		
Parameter	n	a	−b	n	a	−b
G. mirabilis						
San Francisco	46	36.9	0.186	34	68.6	0.138
Los Peñasquitos	53	43.7	0.277	43	86.2	0.286
Salton Sea	46	31.0	0.160	36	62.6	0.193
San Felipe	35	57.1	0.346	35	79.5	0.247

Temperature	24° C.			31° C.		
Parameter	n	a	−b	n	a	−b
G. mirabilis						
San Francisco	36	254	0.577	36	300	0.526
Los Peñasquitos	49	256	0.560	40	331	0.530
Salton Sea	36	310	0.626	36	419	0.622
San Felipe	34	246	0.568	34	351	0.613
San Carlos	54	248	0.451	42	318	0.487
G. seta						
Puertecitos	24	177	0.289	24	289	0.257

<center>TABLE II</center>

Results of co-variance tests on differences in rate of oxygen uptake in Gillichthys mirabilis *and* G. seta
Entries refer to G. mirabilis *unless stated otherwise.   F = F ratio, d.f. = degrees of freedom,*
$_{0.95}$ *= 5% critical value*

	Tempera-ture	F	d.f.	$F_{0.95}$	Decision
Hypothesis: means same after adjustment for body weight					
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	10° C.	8.50	3,175	2.66	Reject
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	17° C.	6.58	3,143	2.67	Reject
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	24° C.	1.93	3,150	2.67	Accept
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	31° C.	5.46	3,141	2.67	Reject
G. mirabilis (San Carlos)/*G. seta* (Puertecitos)	24° C.	28.6	1,76	3.97	Reject
G. mirabilis (San Carlos)/*G. seta* (Puertecitos)	31° C.	1.07	1,63	3.99	Accept
Hypothesis: Same slope within the groups					
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	10° C.	2.65	3,172	2.66	Accept
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	17° C.	3.58	3,140	2.67	Reject
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	24° C.	0.68	3,147	2.67	Accept
San Francisco/Los Peñasquitos/Salton Sea/San Felipe	31° C.	2.25	3,138	2.67	Accept
G. mirabilis (San Carlos)/*G. seta* (Puertecitos)	24° C.	7.65	1,74	3.97	Reject
G. mirabilis (San Carlos)/*G. seta* (Puertecitos)	31° C.	12.6	1,62	4.00	Reject

the curves in Figure 1, when recalculated in cc. O_2/kg./hr. and plotted on semi-logarithmic graph paper.

For all groups, the mean rate of oxygen consumption remained consistent over a 12-hour period after adjustment, and no cyclic phenomena were evident.   During the readings the rate of uptake of each fish fluctuated, resembling fluctuations noted for other fishes by Spoor (1946) and by Pritchard (1955).

Influence of size on oxygen consumption

As is so often the case (examples in Dehnel, 1960), the smaller the animal, the greater its oxygen consumption per unit weight.   In *G. mirabilis* and in *G. seta* this relationship between size and metabolism is more pronounced than in most fishes, the values of *b* being large (see Discussion).   Moreover, both inter- and intraspecific differences in the estimate of *b* were found in the genus *Gillichthys*.

The slopes of the lines at 10° were nearly the same for the various populations of *G. mirabilis*, though the differences approached the 5% level of significance (Tables I, II).   The slopes for the populations steepened from the northmost (−0.19) to the southmost (−0.35), excluding the Salton Sea group (Table I, Fig. 2), but these differences were of doubtful significance.
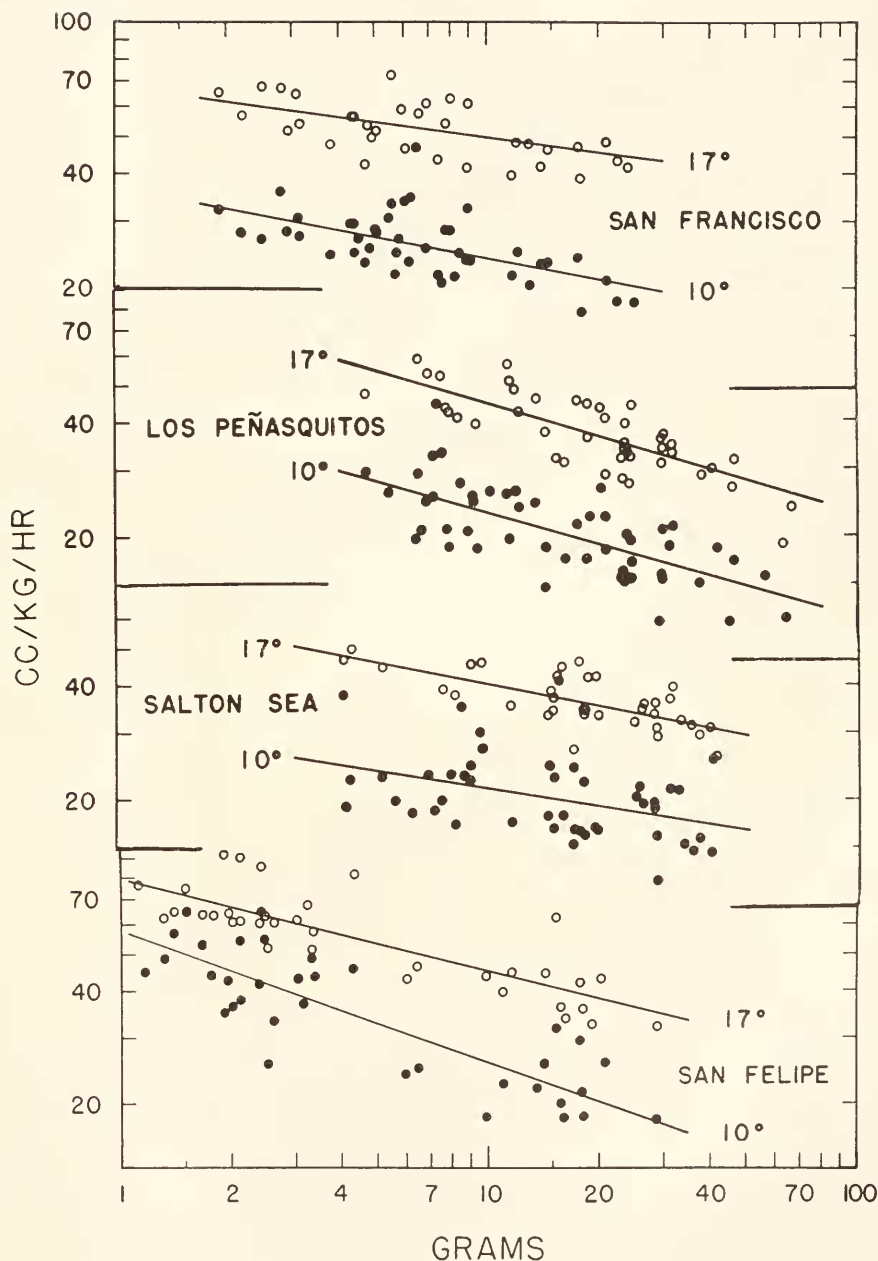
FIGURE 2. Relation between oxygen consumption and body weight of *Gillichthys mirabilis* from San Francisco, Los Peñasquitos, Salton Sea, and San Felipe at 10° (closed circles) and 17° (open circles).
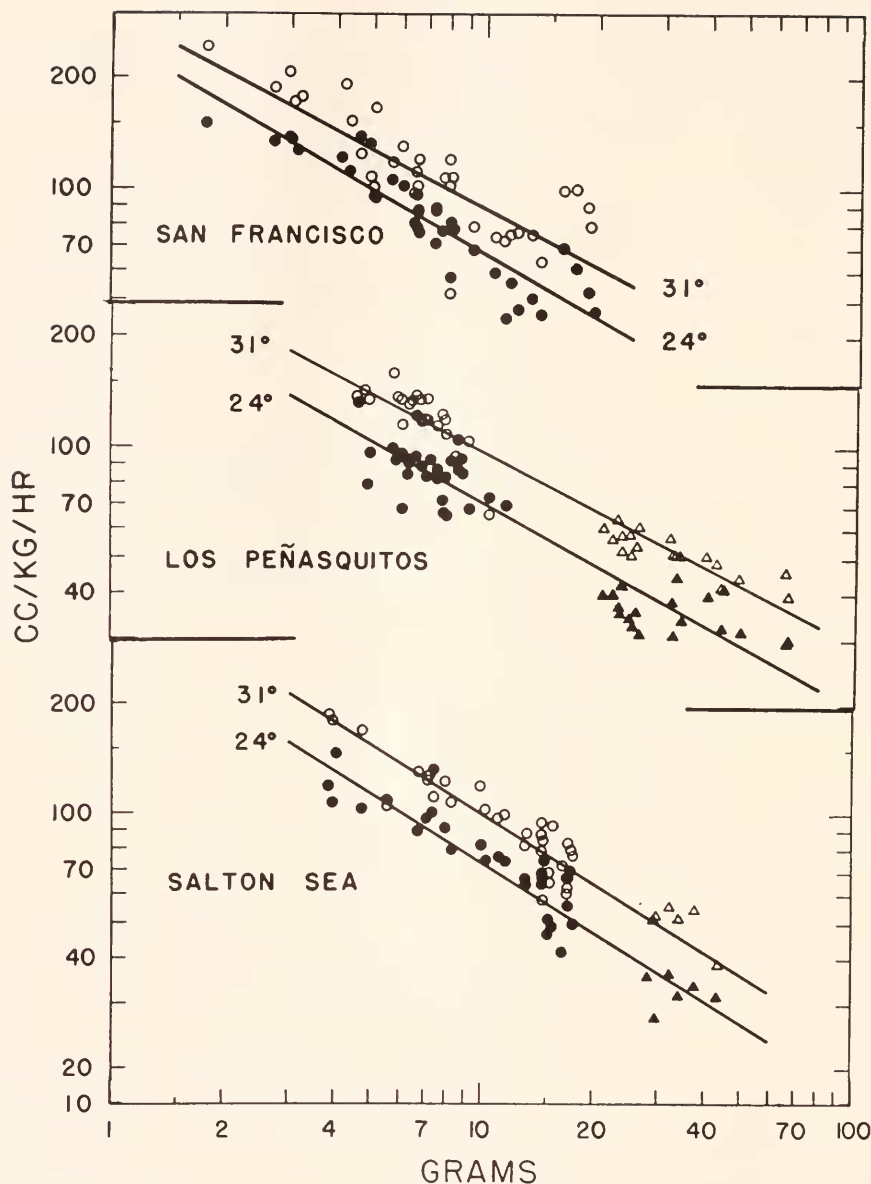
FIGURE 3.   Relation between oxygen consumption and body weight of *Gillichthys mirabilis* from San Francisco, Los Peñasquitos, and Salton Sea at 24° (closed symbols) and 31° (open symbols) ; in small and medium chambers (circles), and in large chambers (triangles).   Chamber size not indicated for San Francisco.

At 17° the slopes for the various populations were significantly different (Table II), though the relationship between them was similar to that observed at 10° (Table I, Fig. 2).   The value of b was low for fish from San Francisco and Salton
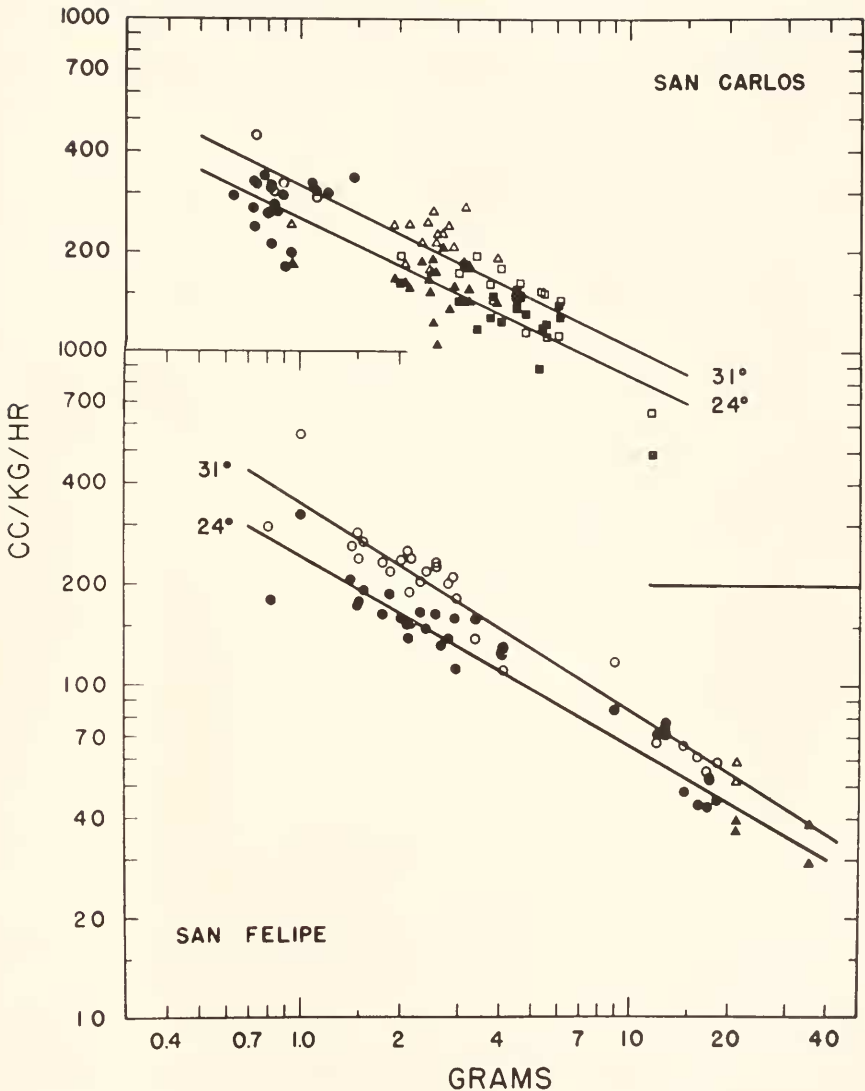
FIGURE 4. Relation between oxygen consumption and body weight of *Gillichthys mirabilis* from San Felipe at 24° (closed symbols) and 31° (open symbols) ; in small and medium chambers (circles), and in large chambers (triangles) ; and of *G. mirabilis* from Puerto San Carlos, at 24° (closed symbols) and 31° (open symbols), in small and medium chambers (not indicated) ; tested on 21 Feb., 1958 (circles), 26 March, 1958 (triangles) and 15 May, 1958 (squares).

Sea, and higher for those from San Felipe and Los Peñasquitos.   Thus the increase in temperature sharpened the differences but did not alter their direction.   No trend with latitude was noted for the slopes at 17°.

The values of the constant *b* were statistically uniform at 24° (Tables I, II;

Figs. 3 and 4). The fish from San Carlos were not included in the statistical analysis, but the slope of their data (-0.45) was lower than that of the four principal populations.

Even the stress of a higher temperature, $31°$, failed to reveal significant differences between the slopes (Tables I, II; Figs. 3 and 4). The value of b for the San Carlos fish (-0.49) diverged from the other groups in the same direction as at $24°$ in that it was lower again.

Comparisons of the slopes obtained from *G. seta* and from *G. mirabilis* (San Carlos), showed that the two species differed at $24°$ and at $31°$ (Tables I, II; Figs. 4 and 5). The separation at $31°$ was particularly noteworthy because at this temperature the mean rates of oxygen consumption (see below) were not significantly different for the two species.

Both at $24°$ and at $31°$, the constant b for young *G. mirabilis* (-0.45 and -0.49) was closer to that of *G. seta* (-0.24 and -0.26) than were the slopes for larger *G. mirabilis* (-0.56 to -0.63, and -0.53 to -0.62). Compared to adult *G. mirabilis,* the slope was lower for smaller *G. mirabilis,* and lower still for *G. seta.*

Mean rates of oxygen consumption

At $10°$ the mean rates of oxygen consumption of the four populations of *G. mirabilis* were significantly different (Table II, Fig. 2); one regression for all the data also was not statistically acceptable (statistical parameters for this test are not indicated in Table II). This divergence apparently was caused by the smaller fish.

Within the experiments at $17°$, the mean rates of oxygen consumption of the four populations were not the same, nor was one regression adequate (Table II, Fig. 2). The divergence, however, seemed to be independent of fish size.

The means of the rates of oxygen consumption at $24°$ (not including the San Carlos sample) showed no differences, and the hypothesis of one regression line was just acceptable (Table II, Figs. 3 and 4). The larger fish from San Carlos fell on about the same regression as did the fish from the four other populations, whereas the smaller San Carlos fish seemed to lie slightly below this regression.

Under stress of a higher temperature, $31°$, differences arose between the mean rates of consumption of the four populations, and one regression also was statistically unsatisfactory (Table II, Figs. 3 and 4). No trend between the means of populations was discernible. The rates of the smaller fish from San Carlos once more appeared to lie below the regression for the other groups.

The oxygen uptake of *G. mirabilis* from San Carlos was compared with that of *G. seta* from Puertecitos. At $24°$ the means were significantly different, but at $31°$ the average rates were the same (Table II).

Influence of temperature on oxygen consumption

Four sets of Q_{10} comparisons were made for respiratory rates. Two categories involved the acute changes $10°$ to $17°$, and $24°$ to $31°$. Another category was the comparison of rates at $10°$ and at $24°$ after acclimation, the chronic changes. Finally, the rates for fish acclimated to $10°$ but tested at $17°$ were contrasted with the rates for fish acclimated to $24°$ but tested at $31°$. These relationships are summarized in Table III. Q_{10} was used only as a descriptive device.

Table III

Temperature coefficients (Q_{10}) for Gillichthys mirabilis *and* G. seta

	10° C. to 17° C.		24° C. to 31° C.	
	1 g.	10 g.	1 g.	10 g.
G. mirabilis				
San Francisco	2.42	2.84	1.27	1.50
Los Peñasquitos	2.64	2.56	1.45	1.59
Salton Sea	2.73	2.45	1.45	1.57
San Felipe	1.60	2.22	1.66	1.43
San Carlos	—	—	1.43	1.47
G. seta				
Puertecitos	—	—	2.01	2.24

	10° C. to 24° C.			17° C. to 31° C.		
	1 g.	10 g.	50 g.	1 g.	10 g.	50 g.
G. mirabilis						
San Francisco	3.97	2.09	1.33	2.87	1.52	0.97
Los Peñasquitos	3.53	2.22	1.61	2.62	1.75	1.32
Salton Sea	5.17	2.40	1.41	3.89	1.92	1.17
San Felipe	2.84	1.97	1.52	2.89	1.58	1.04

Rates used in the calculations of the Q_{10} values were obtained from intercepts of the regression lines. Most comparisons were made at the 10-gram intercept because this value was near the median of the size ranges for most samples. The temperature coefficients at the one-gram intercept, too, were listed to facilitate comparisons with other published results. Vagaries of the slope due to chance can be magnified by comparisons made outside the size distribution of the fitted lines, and may lead to erroneous conclusions.

When the temperature was raised from 10° to 17°, the Q_{10} of fish acclimated to 10° had a value greater than 2.0 in all populations at the 10-gram level. This was appreciably higher than the Q_{10}'s recorded for animals acclimated to 24°.

The Q_{10}'s for *G. mirabilis* (10-gram fish) from San Felipe were almost always the lowest for that species. The other Gulf population of *G. mirabilis,* from San Carlos, also had a commensurately low coefficient for the one temperature interval at which it was tested. Thus, the *G. mirabilis* from the Gulf of California had lower temperature coefficients than those from the Pacific Coast, or Salton Sea.

Within the north to south sequence of populations in California (San Francisco, Los Peñasquitos, and Salton Sea), the predominant trend was higher coefficients to the south. But for the acute change in temperature among the 10° acclimation groups, the trend was just the opposite. In general, the values for Los Peñasquitos and Salton Sea were closer to one another than to that for San Francisco, particularly for acute changes.

Gillichthys seta clearly had a higher temperature coefficient than any population of *G. mirabilis* at 24° (Table III). No *G. seta* were tested at 10°.

An additional set of experiments was performed to establish whether or not the

high chronic Q_{10}'s observed in the cold-acclimated fish could be ascribed to the removal of cold depression. The three populations of *G. mirabilis* from California acclimated to $10°$ were tested at $3.5°$ intervals from $10°$ to $27.5°$. The program was the same as described in Material and Methods.

Cold depression was not a factor in the temperature response of the cold-acclimated fish. As seen in Figure 6, the Q_{10}'s remained approximately the same between $10°$ and $24°$.

Prosser (1958) has presented a classification of types of metabolic responses to temperature changes in relation to cold- and warm-acclimation. To provide such a comparison, data from a warm-acclimated ($24°$) group of *G. mirabilis* from San Francisco were included in Figure 6. This curve, however, has been improvised from two different experiments, and requires an explanation.

The points representing rate of oxygen consumption at $24°$ and $31°$ were derived from the lines fitted to the results of the standard experiment (Fig. 3), and are reliable.

The values for $17°$ and $34.5°$ were obtained as follows. The fish in the respirometers were slowly (eight hours) cooled from $24°$ to $17°$, held at $17°$ four hours, recorded two hours, then tested at $3.5°$ intervals up to $34.5°$, following the usual program. The values at $17°$ regularly fell near the projected $24°–31°$ line. Warming the water to $20.5°$ and $24°$, on the other hand, resulted in pronounced individual differences. These seemed to be of two types: (1) some of the fish manifested high Q_{10}'s, followed by a decrease in rate at a higher temperature, then an increase again at a still higher temperature, whereas (2) the others showed what could be called the anticipated regular increment. I have exercised my judgment and selected out those data that I believe to be reliable, *i.e.*, all the recordings from the San Francisco fish taken at $17°$ and $34.5°$, but none in between.

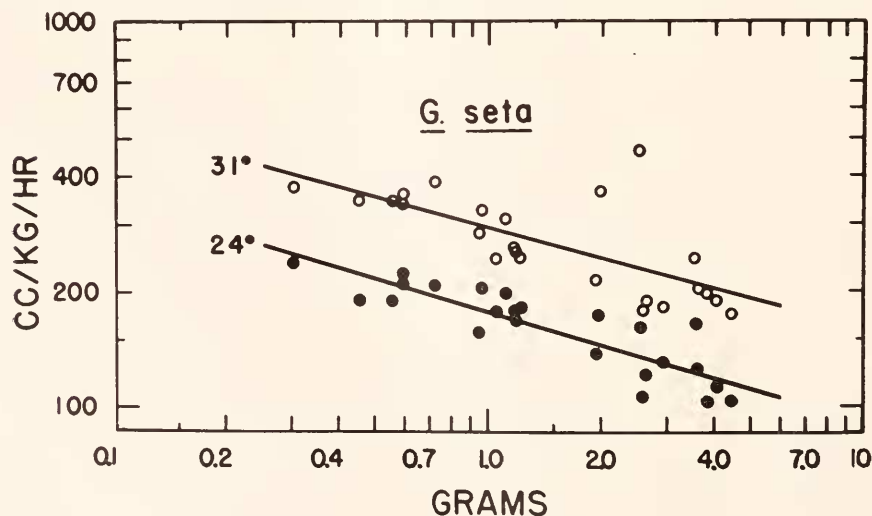The change in Q_{10} revealed by a comparison of the two kinds of curves in Figure

FIGURE 5. Relation between oxygen consumption and body weight of *Gillichthys seta* from Puertecitos at $24°$ (closed circles) and $31°$ (open circles).
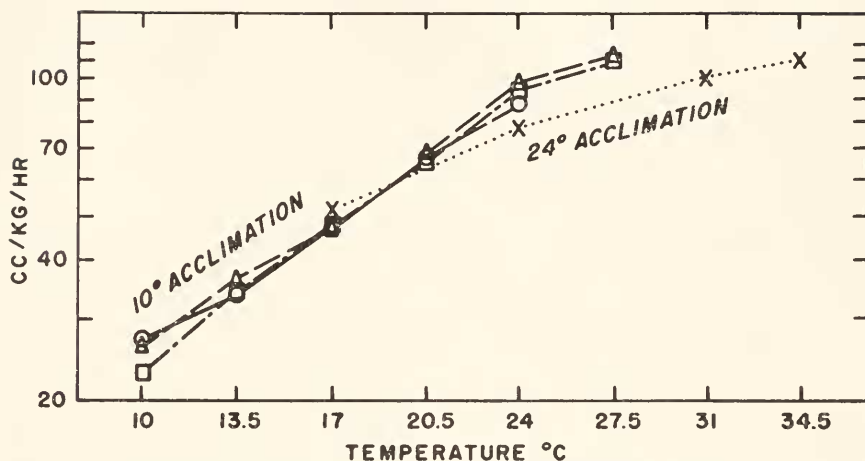
FIGURE 6. Influence of cold- and warm-acclimation on metabolic responses of *Gillichthys mirabilis* to temperature changes. Each point represents the mean rate of oxygen consumption for ten fish, weighing from 4 to 10 grams. Individual rates were adjusted to the 8-gram intercept using the appropriate values of *b* listed under 10° and 24° in Table I; the procedure is discussed in Roberts (1957). Acclimation to 10°: circle = San Francisco population, triangle = Los Peñasquitos population, square = Salton Sea population. Acclimation to 24°: X = San Francisco population.

6 would be classified in the system proposed by Prosser (1958) as Type III B, rotation counter-clockwise with cold acclimation. Clockwise rotation evidently is more common than counter-clockwise (Prosser, 1958). The curves for warm- and cold-acclimated fish intersect between 17° and 20.5°. The curve (not shown) for the warm-acclimated fish from Los Peñasquitos intersects the curves for cold-acclimated animals at 20.5°.

Evidently little or no translation is involved. This would be more apparent if the warm-acclimated fish had been tested over a lower temperature range. Incidentally, above 34° the oxygen uptake in many of the 24°-acclimated fish decreases, and between 35° and 37° many die (further experiments not reported here).

Influence of size on temperature coefficients

There was no apparent difference in Q_{10} between large and small *G. mirabilis* for acute temperature changes. But the temperature coefficients after acclimation were influenced greatly by the size of the fish involved. The chronic Q_{10}'s were strikingly higher in the smaller fish (Table III; Figs. 2 to 4). Apparently acclimation was more effective in larger fish. The 50-gram fish, acclimated to 24° and tested at 31°, commonly had lower respiratory rates than fish of the same size acclimated to 10° and tested at 17°. The size of the fish, therefore, is important in chronic, but not acute, metabolic adjustments.

DISCUSSION

Evidence in fishes for intraspecific differentiation of adaptive physiological systems has received infrequent but notable attention. Heuts (1947, 1956) described

the physiological adaptations to salinity and temperature in the embryos of three races of the stickleback, *Gasterosteus aculeatus*. Minamori (1957, and earlier papers cited there) studied in detail various aspects of developmental physiology in races of the spinous loach, *Cobitus taenia*. The races were arranged in a series according to temperature adaptation. The forms ecologically adapted to higher temperatures, tolerated higher temperatures and developed more slowly at intermediate temperatures than did the cold-adapted forms. Hart (1952) investigated geographic variation in heat tolerance in twelve species of fresh-water fishes. In three species, only, significant differences were apparent between subspecies. In field experiments, Wohlschlag (1957) discovered the sympatric, but ecologically separate forms of an arctic whitefish, *Coregonus sardinella*, had different rates of oxygen consumption. But these "forms" of *Coregonus* probably would be considered distinct species by Svärdson (1953). Guppies, *Lebistes reticulatus*, from inbred lines had different susceptibilities to high temperatures (Gibson, 1954). Under extreme environmental conditions some genotypes of carp, *Cyprinus* (sp.?), were more viable than others (Kirpichnikov, 1945).

Differences between populations, after acclimation, usually are attributed to genetic dissimilarities. The evidence for this thesis is indirect, but consistent (references in Introduction, and Fry, 1957a). The role of the environment in modifying the degree of reversibility, however, is not known.

The temperatures associated with early development allegedly can alter the thermal resistance of guppies, *Lebistes reticulatus* (Gibson, 1954). The thermal resistance of fish reared at 25° and at 30° was similar, but those cultured at 20° were less resistant. At the lowest temperature only two female guppies could be induced to give birth, and at the highest temperature mortality was appreciable. The fish reared at the high, and especially at the low, temperatures could have been selected for by the effects of the thermal environment. Other experiments in the same paper demonstrated different heat resistances in different genetic lines. Males and females also had dissimilar tolerances, and the sex ratios in the experiments on thermal history were not given. The influence of the early thermal experiences on the metabolism of adult poikilotherms remains, then, an open question.

The results reported here, nonetheless, are interpreted as though metabolic differences between fully acclimated animals from naturally occurring populations, indicate heritable dissimilarities.

Influence of size on oxygen consumption

Although the regression coefficients relating oxygen consumption to weight were not especially informative at the intraspecific level, they were most revealing for species relationships. The slope of the regression for the small species, *G. seta*, obviously was lower than that for the adults of *G. mirabilis* (24° acclimation). The disparity was less apparent when the data from adult *G. seta* were compared with those for sub-adult *G. mirabilis*. As noted below, the value of b (*i.e.*, $-b$) tended to decrease at small sizes in *G. mirabilis* (acclimated to 24° only). Morphologically, adult *G. seta* retain many characteristics which in *G. mirabilis* are juvenile (Barlow, 1961). The low regression coefficients for oxygen uptake in *G. seta* might also represent a retention of a juvenile feature.

Von Bertalanffy (1951) discussed the relation between regression coefficients

and phylogeny. He opined that some higher taxonomic categories could be characterized by the value of b. Apparently this does not hold for gobiid fishes since the slopes differ between species, and change with acclimation as well.

Certain difficulties were encountered with the data obtained from fish acclimated to 24°. The relationship between the logarithms of oxygen consumption and body weight was not strictly rectilinear over the size range of the experimental animals. There was a tendency for the negative value of b to decrease slightly in the smaller *G. mirabilis*, and also in the large adults, though less so. As Zeuthen (1955) suggested, it may be best to regard b as a tangent to some more complicated curve.

To complicate matters, the value of b in *G. mirabilis* acclimated to 10° was relatively constant over the entire size range of the experimental animals. The low temperature of 10° is a normal winter temperature for *G. mirabilis*, but surely it is suboptimal for this species in terms of activity and growth; the fish seem well suited to 24°. Possibly, the low temperature of 10° effects a physiological compensation for the unnatural continuous illumination. Or, at 10° metabolism is less affected by growth and activity. The values of b obtained from the cold-acclimated fish are more in accord with most of those reported for other fishes.

Zeuthen (1953) suggested that -0.22 probably is a representative value for b among fishes. Fry (1957b) cited -0.10 to -0.20 as usual values for b, but also noted five instances where values of from -0.33 to -0.50 had been reported. The value of b, -0.50 to -0.60 for *G. mirabilis* acclimated to 24°, then, is higher than that usually considered typical for fishes. The value of b (-0.15) reported by Scholander *et al.* (1953), was derived from a composite of several species. The data for some of the species, however, appear to show a slope greater than -0.15 (*Abudefduf saxatilis, Scarus croicensis*). Wohlschlag (1957) recorded a slope of -0.60 for a coregonid fish, though he attributed the high value to the small size of the sample. Recent investigations into other animal groups have revealed values of b well above -0.33 predicted from the surface volume relationship (references in Zeuthen, 1953; Mann, 1956; Dawson and Bartholomew, 1956; Vernberg, 1959a; Dehnel, 1960; Velma Vance, personal communication).

Mean rates of oxygen consumption

Physiological responses to stress may reveal differences when measurements within the temperature range to which the animals are adjusted might fail (Prosser, 1957). The means of the rates of oxygen consumption in *G. mirabilis* were the same only at 24°, which probably lies within its optimal temperature range. Differences were apparent when the temperature was raised to 31°. The lowest temperature, 10°, must have been stressful: activity was greatly reduced, although social behavior, frontal display by males, was observed. Fish acclimated to 17° were only slightly less active than those at 24°. Nonetheless, the sudden 7° increase in temperature from 10° to 17° probably stressed the metabolic system of the cold-acclimated fish.

While differences in mean rates of oxygen consumption at a given temperature appeared to indicate divergences between populations of *G. mirabilis*, the direction of the departures seemed to be irregular. At 10°, only, there may have been evidence for higher metabolic rates to the south in smaller fish. This was particularly noticeable in the animals from San Felipe, that had been tested in late spring. The

other populations had been examined during the winter. But if there had been a residual seasonal adjustment, the rates for the San Felipe group should have been low in comparison to the winter groups. As seen, just the opposite occurred.

The oxygen consumption of *G. mirabilis* is similar whether the fish are in or out of water. Though not measured in water, the rates reported here for aerial respiration of *G. mirabilis* agree with those found by Wells (1935a) for respiration in water of the same species, when allowances are made for differences in thermal acclimation. The rate of oxygen uptake of the Hungarian mud-minnow, *Umbra lacustris* (*krameri?*), also is the same whether entirely aquatic, or a combination of aquatic and aerial (Geyer and Mann, 1939).

The rates of oxygen uptake in *Gillichthys mirabilis* and *G. seta* accord with the values reported for other species of air-breathing Gobiidae. The rates at 24.5° for *Periophthalmus vulgaris* (seven grams) and *P. dipus* (16 and 17 grams) given by Schöttle (1931) coincide nicely with the regression relating oxygen consumption to weight for *G. mirabilis*. The rate for *P. schlosseri* (110 grams) was about 100% higher than would have been expected for *G. mirabilis* of that size, but agreed well with the extension of the regression for *G. seta*. The data of Schöttle were reduced by 50% before making the comparisons because she recorded the rates during the first hour; the values for *G. mirabilis* decreased by about this amount during the 12-hour adjustment period.

Influence of temperature on oxygen consumption

The responses to temperature changes seemed to be of more adaptive significance than the absolute level of oxygen consumption. While the mean rates showed no interpretable differences, the disposition of the Q_{10}'s suggested a meaningful pattern.

The temperature coefficient data infer the following arrangement of populations: *G. mirabilis* from the north and central regions of the Gulf of California are alike, but differ from the other groups considered by having consistently lower Q_{10}'s; *G. mirabilis* from San Francisco Bay are similar to, but somewhat apart from, the other two populations in California in that their Q_{10} is highest at low temperatures, but relatively low at high temperatures; *G. mirabilis* from the Salton Sea and Los Peñasquitos show a correspondence when responses to acute changes are compared, but are dissimilar when temperature coefficients of acclimated rates are considered.

Temperature coefficients generally are higher with adaptation to higher temperatures (but see Dehnel, 1960): northern species or forms usually have lower coefficients than their southern counterparts (Rao and Bullock, 1954; Minamori, 1957; Heuts, 1956; Tashian, 1956; Dehnel, 1955). In some respects *G. mirabilis* was just the opposite: warm-acclimated fish had low Q_{10}'s in all the groups, and cold-adapted ones had high Q_{10}'s. A further inconsistency was that the Q_{10}'s were higher in northern populations when the fish were cold-acclimated. Certain data, however, were in accord with the hypothesis of higher Q_{10}'s in more southern populations. Warm-acclimated *G. mirabilis* from the Pacific Coast and Salton Sea had higher Q_{10}'s to the south. But the two Gulf of California populations had even lower coefficients than the northmost Pacific Coast population, San Francisco. Certainly the Gulf habitats experience much higher maximum temperatures and a greater range, too, than do those in San Francisco Bay. The temperature patterns in the Gulf and on the Pacific Coast are not equatable.

The most obvious assumption is that the low sensitivity to temperature changes observed in *G. mirabilis* from the Gulf of California is an adaptation to the more variable climate there. Yet *G. seta* from the upper Gulf lives in a habitat subject to even greater thermal oscillations; and the Q_{10}'s for *G. seta* are higher than for any population of *G. mirabilis* under the same conditions. This may not be a fair comparison because it involves another species with different behavior and ecology.

The adaptive significance of the nature of the Q_{10}'s in *G. mirabilis*, and in *G. seta,* cannot be appreciated until more information is at hand, both with regard to the general and the specific aspects of the problem. It might be presumed, though, that within the species *G. mirabilis* low sensitivity to temperature change is an adaptation to higher temperatures, and high sensitivity an adjustment to lower temperatures. This is at variance with the conclusions of Scholander *et al.* (1953) which were based on interspecific comparisons, and also with the hypothesis put forth by Rao and Bullock (1954).

Influence of size on temperature coefficients

The few available data for fishes indicate that size affects Q_{10} in different ways depending on the particular species, and probably on the experimental procedures. The temperature coefficient was said to be independent of size in goldfish, *Carassius auratus* (Fry and Hart, 1948). Within a given race of the loach, *Cobitis taenia*, temperature coefficients of embryos were independent of egg volume (Minamori, 1957). At low temperatures size had no role in the determination of Q_{10}'s in the killifish, *Fundulus parvipinnis*, but the smaller fish were more sensitive at higher temperature intervals (Wells, 1935b; re-evaluated on the basis of relative changes). In another cyprinodontid fish, *Crenichthys baileyi*, from a warm spring, small individuals were more sensitive to a temperature drop than were large ones (Sumner and Lanham, 1942). In the brook trout, *Salvelinus fontinalis*, size had little influence on the response to chronic changes in temperature when standard metabolic rates were compared, except at the highest temperature interval where small fish were more responsive. When active, however, the smaller trout had higher temperature coefficients than the larger ones (Job, 1955). Measured by opercular rates, large carp, *Cyprinus carpio*, were more responsive to long term (chronic) temperature changes than were small individuals (Meuwis and Heuts, 1957).

In *Gillichthys mirabilis*, and in *G. seta*, temperature coefficients were not size-dependent in short term (acute) temperature changes. The relatively greater increase in respiratory rate of small *G. mirabilis* acclimated to 24° as contrasted to 10° may indicate that large *G. mirabilis* are metabolically more homeostatic than small, though only when allowed time to adjust. Meuwis and Heuts (1957) noticed a similar relationship in the carp, except the smaller fish, not the larger, were more homeostatic.

Comparison of physiological and morphological findings

The two species involved, *G. mirabilis* and *G. seta*, are distinct but difficult to distinguish from one another (Barlow, 1961). Ichthyologists, unless experienced with this group, frequently have difficulties separating them. In many respects *G. seta* represents an extension of the morphological cline manifested by the widely

distributed populations of *G. mirabilis* (Barlow, unpublished data). Physiologically, however, the two species diverge where their distribution overlaps, the Q_{10}'s and the regression coefficients, *b,* showing marked character displacement.

Within the species *G. mirabilis,* the greatest structural differences between populations were for the most part discernible only by means of statistical analysis. The aberrant Salton Sea population, of course, was an exception, but need not disturb the thesis being put forward here. Furthermore, the morphological investigation involved the analysis of many characters, such as measurements of several body parts, counts of the various fins and the vertebrae, and extent and nature of squamation. Among the multitude of possible physiological parameters, only oxygen consumption was studied. Considering this, it is fair to say the magnitude of intraspecific variation in oxygen uptake was commensurate with that observed in the morphological study.

The pattern of divergence, the "species structure" of *G. mirabilis,* became apparent only when many intervening populations, in this case 17, were compared morphologically. It is questionable if much sense could have been made of the morphological data from only those five populations analyzed experimentally. Indeed, before the series of populations had been investigated, three nominal species had been proposed for the two under discussion, and the situation was not at all clear (Barlow, 1961).

Beyond this, it might be pertinent to ask if the experiments were designed properly to reveal meaningful differences in the respiratory rates. Perhaps more informative data could be obtained by acclimating the animals to a controlled regime of oscillating temperatures, simulating the natural situation. After all, the most obvious thermal dissimilarity in the locales involved is the range of temperatures experienced by each, not the mean. Constant temperature, not to mention illumination, is an unreal circumstance for the animals; the absence of these modulating influences might even be inimical to certain metabolic processes.

In closing, the lack of differences in the nature of the oxygen uptake at 24° should be emphasized. From numerous field observations, I believe this temperature is near the thermal optimum for the species *G. mirabilis* throughout its geographic range. The similarities at 24°, therefore, might have been expected. The dissimilarities in the populations were manifested as the temperature departed from this value. The environment of each population evidently is unique in its combination of various temperatures (especially maxima and minima), photoperiod, salinity, and so on. Probably the singular features of each habitat have stamped their character on the gene pool of the population, effecting a slight, though significantly adaptive, divergence in the metabolic response to temperature.

Summary

1. Rate of oxygen uptake was determined for moist fish in a Warburg apparatus. Fish acclimated to 10° were tested at 10° and 17°; those acclimated to 24° were tested at 24° and 31°. Five populations of *G. mirabilis* were investigated (San Francisco Bay in central California, Los Peñasquitos in southern California, San Felipe in the northern Gulf of California, Puerto San Carlos in the central Gulf, and the Salton Sea). One population of *G. seta* (from the upper Gulf) was studied at 24°.

2. Rate of oxygen consumption per unit weight was plotted against body weight. The regression coefficient, *b*, differed between populations for *G. mirabilis* though not in an interpretable fashion. The value of *b* was low at 10° and 17° (−0.14 and −0.35), and high at 24° and 31° (−0.45 and −0.63). The slopes were much lower for *G. seta* at 24° and 31° (−0.29 and −0.26) than for *G. mirabilis*.

3. The mean rates of oxygen uptake were different for the populations of *G. mirabilis* at all temperatures except 24°. The average rate for *G. seta* at 24° was lower than that for *G. mirabilis*, but the rates were about the same for both species at 31°.

4. The temperature coefficients, Q_{10}, varied from population to population of *G. mirabilis* in what seemed to be a pattern. The coefficients were lower in groups from the Gulf of California at almost all temperature intervals. Within the Pacific Coast and Salton Sea populations, the Q_{10}'s for the northern population were highest at the low acclimation temperature, and lowest at the high acclimation temperature. For all groups, the Q_{10}'s were highest when cold-acclimated (2.2 to 2.8), and lowest when warm-acclimated (1.4 to 1.6). The coefficient for warm-acclimated *G. seta* was high (2.0).

5. There was no apparent difference in temperature coefficients between large and small *G. mirabilis* for acute temperature changes. The smaller fish manifested appreciably higher Q_{10}'s than did the larger fish, however, when rates were compared after acclimation.

6. The degree of intra- and interspecific differences in the nature of the oxygen consumption was roughly of the same order of magnitude as the morphological differences reported in another article.

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